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HORMONAL INFLUENCES IN LEARNING:

The pituitary-adrenal system, anxiety, and avoidance learning

by

Mortimer H. Applezweig and George Moeller

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Norman Applezweig, Director, Biological Derivatives, Inc., New York, served as informal consultant to the project at its outset and supplied some of the hormones used in these studies.

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\* Mr. Franczak was killed in an accident in August, 1953.

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To my wife, who collaborated on one of these projects and who worried with me through all of them, and to George Moeller, the co-author of this report, who was primarily responsible for the statistical analysis of the data of these projects, the largest debt is owed.

M. H. A.

# TABLE OF CONTENTS

Project Personnel . . . . .	1
Acknowledgements. . . . .	11
INTRODUCTION . . . . .	1
PART I: RESULTS, 1951-1952 . . . . .	2
Experiments 1 and 2. . . . .	2
Experiment 3 . . . . .	7
Experiment 4 . . . . .	10
Experiment 5 . . . . .	10
Experiment 6 . . . . .	11
PART II: RESULTS, 1952-1953 . . . . .	14
Experiment 7 . . . . .	14
Experiments 8, 9 and 10 . . . . .	15
Experiment 11. . . . .	23
Experiment 12. . . . .	24
Experiment 13. . . . .	28
Experiment 14. . . . .	29
Experiments 15 and 16 . . . . .	32
Experiment 17. . . . .	32
Experiment 18. . . . .	34
DISCUSSION OF RESULTS . . . . .	36
A. Food and Water Consumption . . . . .	36
B. Activity . . . . .	36
C. Animal Weight. . . . .	37
D. Emotionality . . . . .	38
E. Escape Learning . . . . .	38
F. Fear Conditioning . . . . .	38
1. Adrenalectomized animals . . . . .	38
2. Hypophysectomized animals. . . . .	39
G. Effects of exogenous ACTH administration	39
1. Hypophysectomized animals. . . . .	39
2. Normal animals . . . . .	40
GENERAL DISCUSSION . . . . .	41
SOME ALTERNATIVE HYPOTHESES . . . . .	43
1. The pituitary-adrenocortical system is necessary for avoidance learning . . . . .	43
2. Hypophysectomy produces general debility	44
3. Alternate pathways . . . . .	45
SUMMARY AND CONCLUSIONS . . . . .	46
REFERENCES. . . . .	48

## Introduction

Applezweig and Baudry (1), in 1950, hypothesized the necessity of an intact pituitary-adrenocortical system for the acquisition of an instrumental avoidance response on the basis of the established role of this system in the response of the organism to stress (Selye, 15, 16) and the propositions regarding the necessity of "anxiety" as a mediating condition in the acquisition of avoidance conditioning (Mowrer, 10, 11).

Testing this hypothesis in two pilot experiments with hypophysectomized rats in a Miller-Mowrer shuttle box (12), it was shown that these animals were significantly inferior to a normal control group in such avoidance learning, and that exogenous administration of adrenocorticotrophic hormone (ACTH) appeared to improve this learning. From a comparison of escape and avoidance behavior in these animals, the conclusion was drawn that "...the pituitary-adrenocortical system is involved in the organism's capacity to anticipate painful stimulation, rather than respond to its presence (1)."

During the two year period following this work, the senior author and his associates, with the support of the Office of Naval Research, have been attempting to extend and clarify the findings reported on the role of this particular endocrine system in avoidance learning.

## Part I<sup>1</sup>

### Experiments 1 and 2.

During the first year of this research, two studies were made of food and water consumption and activity levels of groups of hypophysectomized, sham-hypophysectomized, adrenalectomized, and normal rats. Animals were housed individually and measures of food and water intake were taken at fixed times of day once during each 24-hour period.

Normal, intact animals were found to consume more food<sup>2</sup> than did the operated groups, except during the last four days of the experiment when food intake for the adrenalectomized animals increased abruptly to equal that of normals. The food-intake curve of the sham-hypophysectomized animals was depressed a roughly constant amount below that of the normals, except on the first day, when the amount eaten by this group was much less than that eaten by the normals, and on the second day, when they ate slightly more than did the normals.

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<sup>1</sup> Part I is a revision of the first annual progress report of September, 1952, and represents the portion of the research completed while the senior author was at Wesleyan University.

<sup>2</sup> Big Red Dog Food in pellet form was used in these studies with no supplement of any sort (excepting tap water).

The hypophysectomized animals ate less than did any of the other groups on the first two and last three days of the study. On days 3 through 6 they continued to eat less than the normals, but about the same amount as did the other operated groups (see Figure 1).

Hypophysectomized animals drank more water, on the average, than did normal or sham-operated rats, although this difference disappeared in a second study. Adrenalectomized rats consumed more liquid than did any of the other groups in both studies, but they were actually offered and drank only a physiological saline solution (see Figure 2).

Activity level was measured by placing each rat in a rotating activity cage for a one-half hour period daily and counting the number of cage revolutions. Time of day was randomized (within the 3-hour total period used) as was the particular activity cage used for each animal. As can be seen in Figure 3, hypophysectomized rats seemed to give consistently fewer responses, on the average, than did any of the other groups. However, the variation of animal responses from day to day and from rat to rat in all four groups was so great as to obviate statistical significance for any of the comparisons but days for all groups combined. Activity increased from beginning to the end of the observation period.

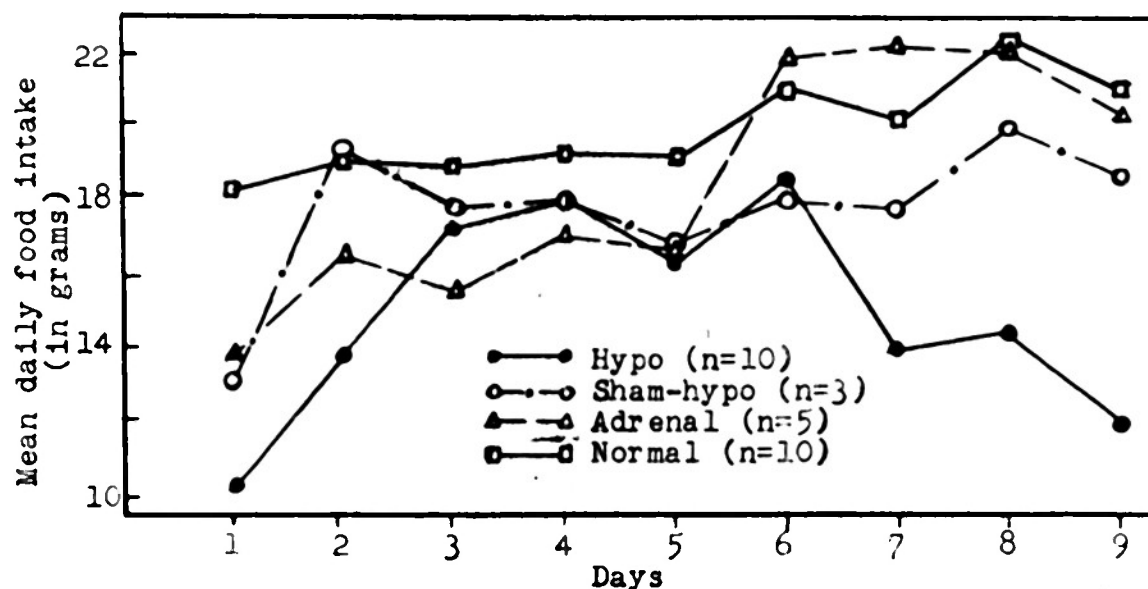


Figure 1. Mean daily food consumption (in grams) during the nine day study period (Experiment 1).

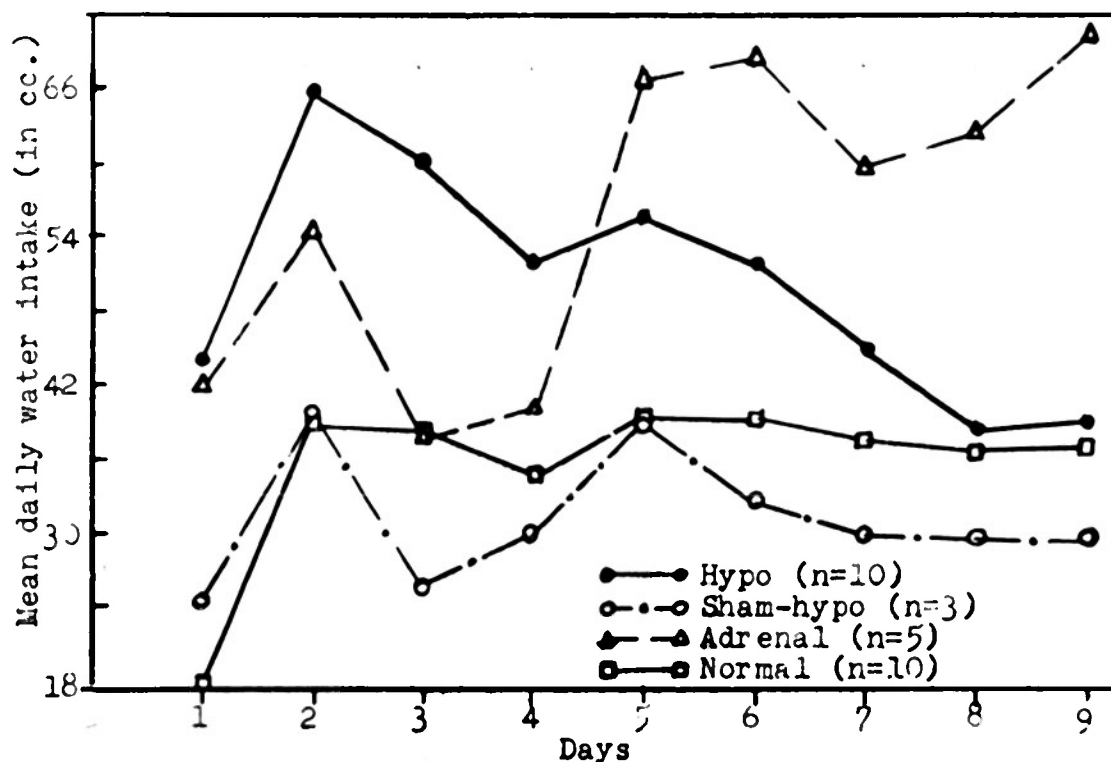


Figure 2. Mean daily water intake (in cc.) during the nine day study period (Experiment 1).



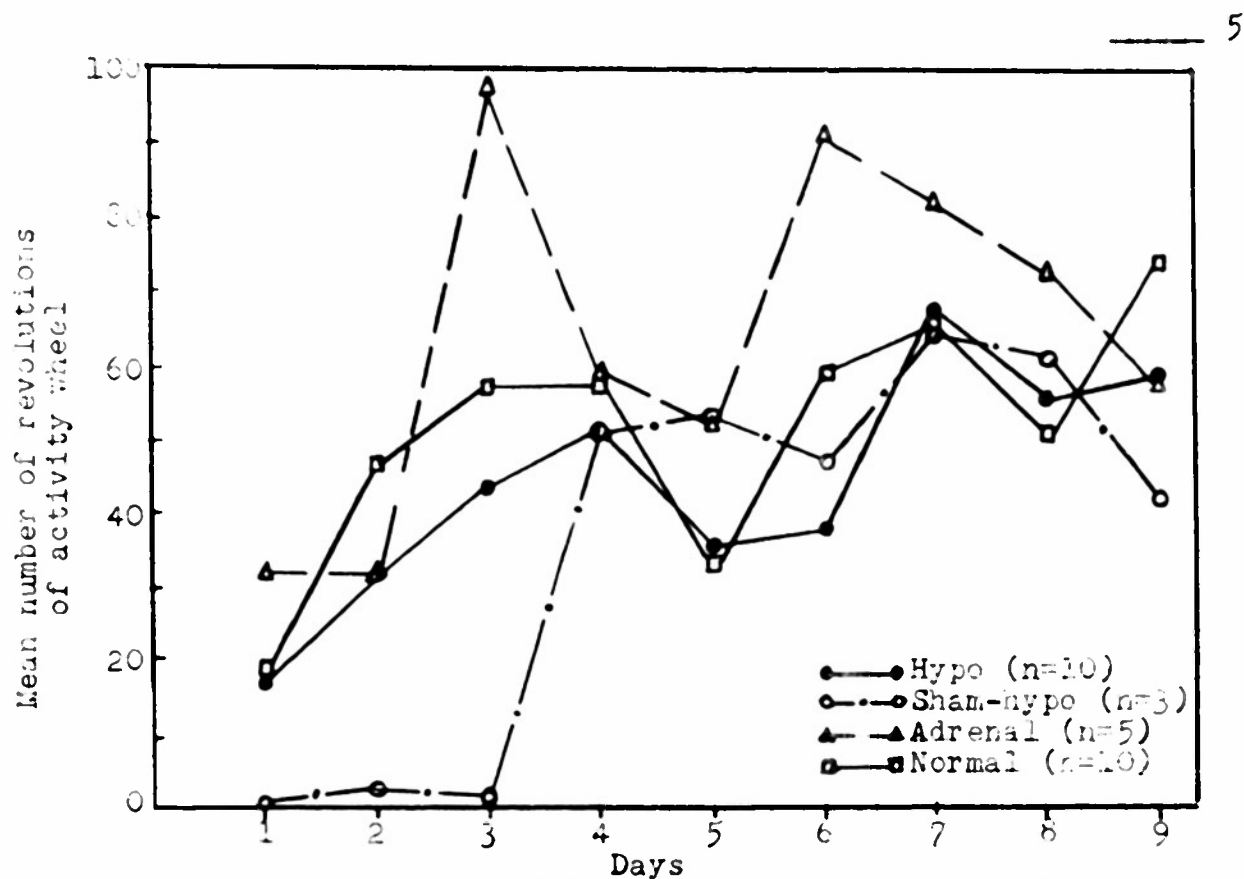


Figure 3. Mean activity during 30-minute activity wheel periods on each of nine study days (Experiment 1).

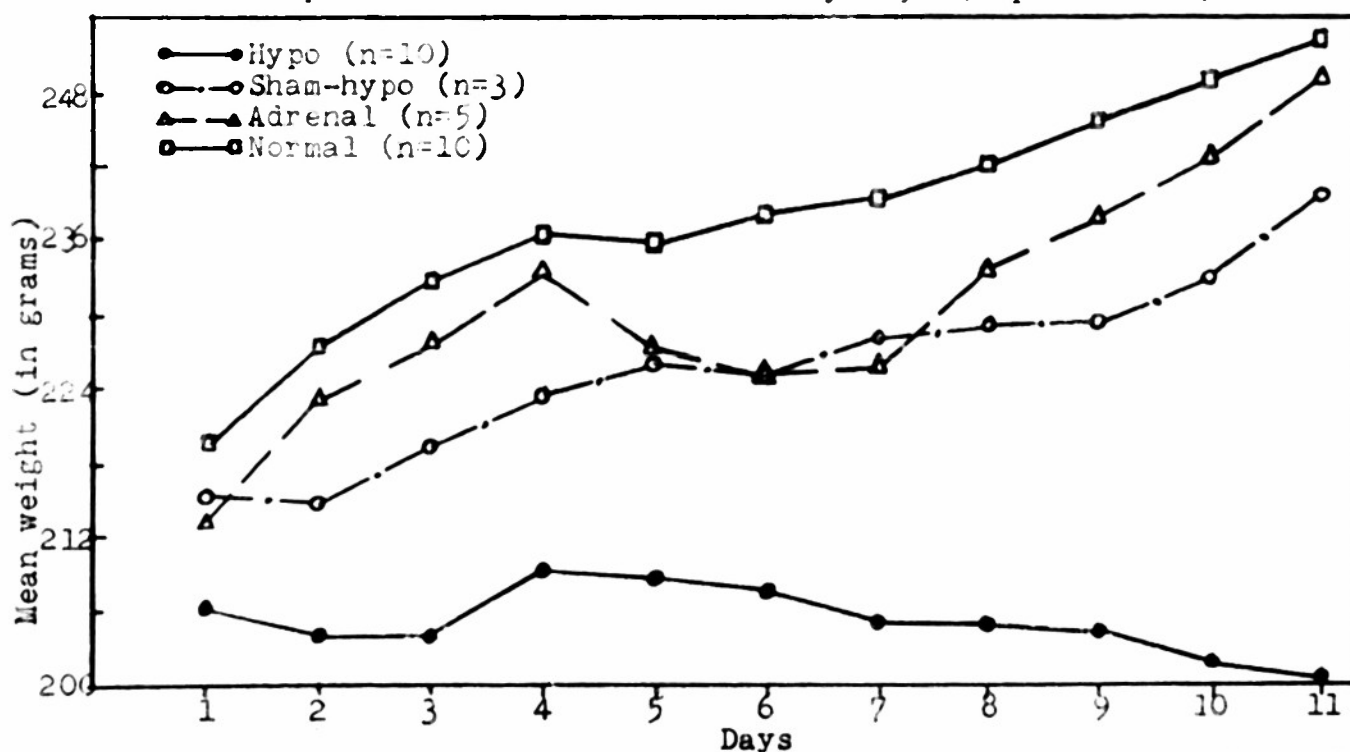


Figure 4. Mean daily weight for 11 days following operation (Exp. 1)

Over the eleven day period of study (following operation) in both series, only the hypophysectomized rats failed to show an increase in weight, with this group actually showing a slight weight loss in one study. Weight gains in the other three groups were of approximately the same magnitude, on the average (see Figure 4). No relationship was found between food and/or water intake and activity level, nor were water intake or activity level found to be correlated with animal weight or weight change. Food consumption and weight change were significantly positively correlated over all groups combined, however.

These studies were undertaken to explore the possibility of studying learning situations involving food and/or water motivation instead of, or in addition to, fear. The results seemed to indicate that the problems of equation of food and/or water motivation and reinforcement for the differently operated groups did not warrant their use at this stage of the present research program. Burnham and Leonard (3), in an earlier study of learning in hypophysectomized rats, were forced to use escape from shock as additional motivation with the insulted animals in order to get them to run through a maze to a food reward. The nature of the present investigations is such that it was thought unwise to complicate them any further with the use of food or water rewards.

Activity level differences, although not statistically significant, were in the direction of the hypothesis of general debility in hypophysectomized animals, a problem which will be dealt with later in this paper.

### Experiment 3.

A repetition of part of the Applezweig and Baudry study (1) of avoidance learning in hypophysectomized and sham-hypophysectomized rats was next attempted. A 2.65-second buzzer preceded a .9 ma shock as CS and UCS, respectively. If the animal responded during the 2.65 seconds the buzzer was automatically turned off and the shock did not occur. If the animal failed to respond by running during this period, shock was applied to the grid floor and continued until the animal responded by crossing the chamber and automatically tripping a floor switch (or until the end of a 22-second period, whichever was the sooner). Animals were forced to cross or be shocked by being dislodged from any position which appeared to permit them to limit or escape from shock without crossing.

The results indicated that both hypophysectomized and sham-hypophysectomized rats were capable of this type of conditioning in the Miller-Mowrer type shuttle box, but in both response latency and the number of trials required to meet the learning criterion, the hypophysectomized group

was significantly inferior to the sham-operated controls (see Figure 5 and Table 1). These findings were in agreement with the earlier study.

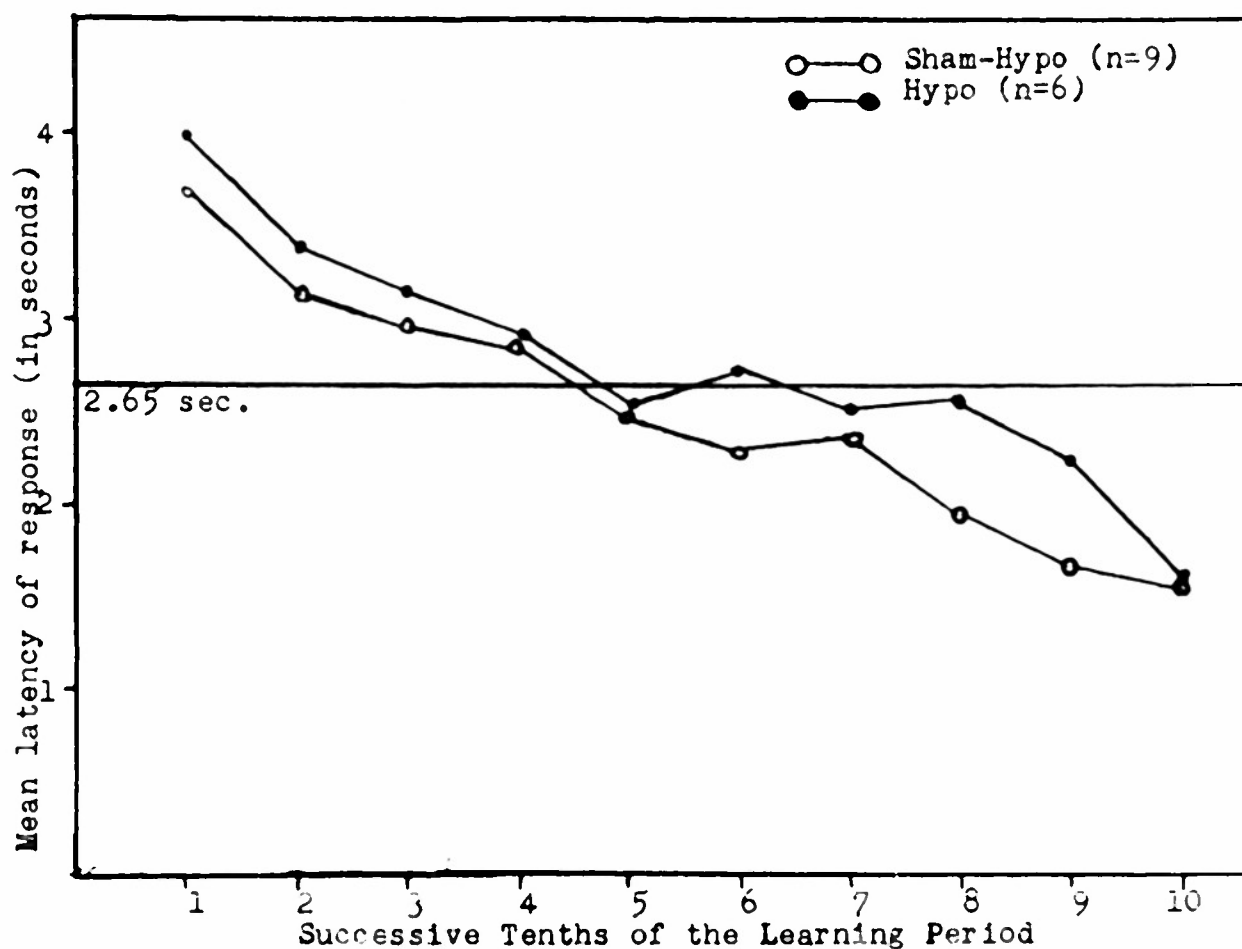


Figure 5. Mean Vincent latency of response. Latencies shorter than 2.65 sec. indicate successful avoidance responses.(Exp.3)

Table 1. Per Cent of Conditioned Responses for  
Daily Blocks of Twenty Trials. (Experiment 3)

Hypophysectomized Rats (n=6)										Sham-Operated Rats (n=9)									
Subj.	1	3	5	19	21	25				2	4	10	12	14	16	18	20	22	
Day																			
1	6	0	25	20	10	10				65	47	42	35	30	17	10	20	45	
2	58	5	37	10	17	38				80	79	18	75	58	61	60	11	58	
3	67	19	60	50	0	40				—	—	55	—	45	95	74	50	75	
4	65	42	80	26	0	65				—	—	74	—	89	—	89	24	—	
5	53	67	—	42	0	60				—	—	95	—	—	—	—	53	—	
6	79	84	—	50	6	53				—	—	—	—	—	—	—	32	—	
7	—	—	—	74	15	60				—	—	—	—	—	—	—	50	—	
8	—	—	—	80	16	85				—	—	—	—	—	—	—	42	—	
9	—	—	—	—	0	—				—	—	—	—	—	—	—	50	—	
10	—	—	—	—	6	—				—	—	—	—	—	—	—	60	—	
Mean	55	36	51	44	7	51				72	63	57	55	56	58	58	39	59	

#### Experiment 4.

In a study of retention of a pre-operatively learned shock avoidance response, normal animals were trained in the Miller-Mowrer box and then divided into two groups paired on performance criteria. One group was then hypophysectomized while the other was sham-hypophysectomized. These groups were then returned to the apparatus and the avoidance response extinguished and then retrained. Unfortunately, a large number of post-operative deaths reduced the number of matched pairs of animals so severely as to make statistical comparison of the results somewhat meaningless. However, the differences were in the predicted direction, with the hypophysectomized rats extinguishing somewhat more rapidly than the matched sham-operated group. These animals were then retrained to the original criterion of avoidance learning and, again, despite the small number of animals in each group, the differences were in the predicted direction, hypophysectomized animals, on the average, taking more trials to reach the learning criterion.

#### Experiment 5.

A study of water-maze escape learning was undertaken to test the hypothesis that the slower learning of hypophysectomized rats was attributable to their general debility rather than to the specific effects of interference with the

pituitary-adrenal system. Hypophysectomized and sham-operated animals were matched for weight and run in a 6-choice water maze (after Ruch, 13). Records of latency, errors, and number of retracings were kept for each pair. The expectation was that the hypophysectomized animals would not be significantly inferior to a control group (as a general debility hypothesis would predict) when the response involved escape from an ever-present noxious stimulus (cold water). Unfortunately, here, too, a large number of animal deaths before the conclusion of the experiment does not permit a definitive statement of conclusion. However, insofar as the data can be analyzed, there appeared to be no differences between the groups in number of errors and/or in number of retracings. The comparative latencies, however, suggest that a difference might be present for this measure, in a direction contrary to the predicted one. The data are far from adequate, however, to permit determination of the issue.

#### Experiment 6.

The final study during this first year was one of activity level and of avoidance learning in four groups of animals with hypophysectomy and ACTH treatment as the two independent variables. Here the evidence of delayed learning in hypophysectomized rats obtained in earlier studies was confirmed. In addition, a group of normal (sham-operated)

and a group of hypophysectomized rats were injected with 1.5 to 2.00 mg. of ACTH each 24 hours for ten days. Injections were given two and one half hours before activity measurement and three hours before shuttle-box training<sup>3</sup>.

ACTH-treated sham-operated normals displayed somewhat greater activity during a ten-day study period, in comparison with the three other groups, but ACTH-treated hypophysectomized animals (treatment begun within 36 hours after operation) could not be distinguished from either their hypophysectomized controls<sup>4</sup> or sham-operated normals in activity level. However, when activity was measured by the number of spontaneous crossings during actual training (between-trial crossings without experimenter-initiated stimulation), the non-ACTH-treated normals were more active (due primarily to two very active rats), with the other three groups indistinguishable from each other. Individual animal variations were extremely large in both types of activity measures. Neither form of activity, incidentally, was found to be correlated with individual speed or adequacy of conditioning.

A comparison of learning data of the four groups (see

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<sup>3</sup> The estimate of a 2½-3½-hour optimal delay for exogenous administration of ACTH is based on inferences from data of Sayers and Sayers (14).

<sup>4</sup> Non-ACTH-treated animals received equivalent injections of .09% saline solution.



Figure 6) failed to confirm findings of the previous year (1) that exogenously-administered ACTH improved rate of learning in hypophysectomized animals. ACTH-treated operated animals did not differ from operated animals not receiving this hormone. ACTH administration to normal animals seems to depress

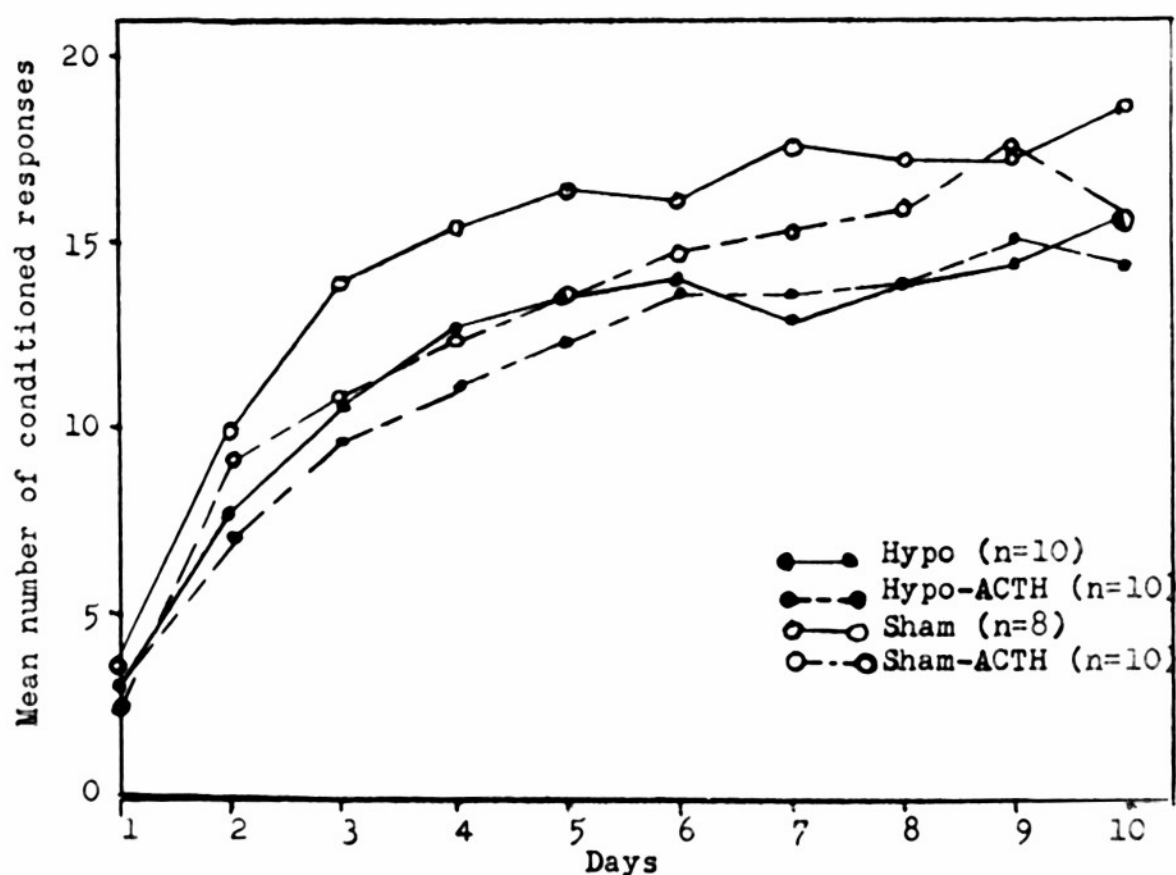


Figure 6. Mean number of conditioned responses for blocks of twenty conditioning trials. (Experiment 6)

the rate of learning somewhat, compared with a non-ACTH normal control group. One obvious difference between the two

studies was the somewhat larger dose of ACTH administered in this study as compared with the previous amount used (.5 mg.). It is possible to speculate that the larger dose served to obscure a facilitating effect.

## Part II<sup>5</sup>

During the second year of this research program, twelve additional investigations were undertaken in an attempt to extend the earlier findings to new experimental situations.

### Experiment 7.

A study of avoidance learning in adrenalectomized rats used the same Miller-Mowrer shuttle box employed in the previous investigations of shock-avoidance in this series. An annunciator-type buzzer was employed as the CS while .9 ma shock was administered through a floor grid as the UCS. The CS-UCS interval was 2.65 seconds. The data showed no differences in either rate of acquisition or level of attainment of avoidance conditioned responses between adrenalectomized and sham-operated groups. Figures 7 and 8 present curves comparing the two groups on mean daily response

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<sup>5</sup> Part II of this report represents the portion of the research completed at Connecticut College.

latencies and mean daily per cent conditioned responses. Animals were run 10 trials per day for 20 days. (Data for adrenalectomized animals include only those animals meeting the "Beach Test"<sup>6</sup> criterion for complete bi-lateral adrenalectomy.) An examination of these figures indicates the complete comparability of the performance of the two groups, confirming earlier preliminary findings of the same nature. A plot of daily medians for the two comparisons (not presented) does not change the picture at all.

#### Experiments 8, 9 and 10.

The next three investigations attempted to extend the earlier findings for fear in the acquisition of avoidance conditioned responses to a study of the acquisition of fear in a classical conditioning situation. Brown, Kalish and Farber (2) have shown that the serial pairing of a neutral stimulus with a noxious stimulus increases the sensitivity of an animal to stimulation as a function of continued pairings. The development of a fear response in connec-

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<sup>6</sup> The "Beach Test" refers to a procedure used by Dr. Frank Beach as a criterion measure of effective complete adrenalectomy. Adrenalectomized animals are maintained on a saline solution for the duration of the experiment and saline is then removed from their drinking water. Completely adrenalectomized animals are unable to survive without physiological saline supplement. Animals in which the adrenalectomy is incomplete or in which auxiliary tissue is able to carry on adrenal function continue to live and grow normally. Animals surviving this return to water for 10 days or more were dropped from this study.

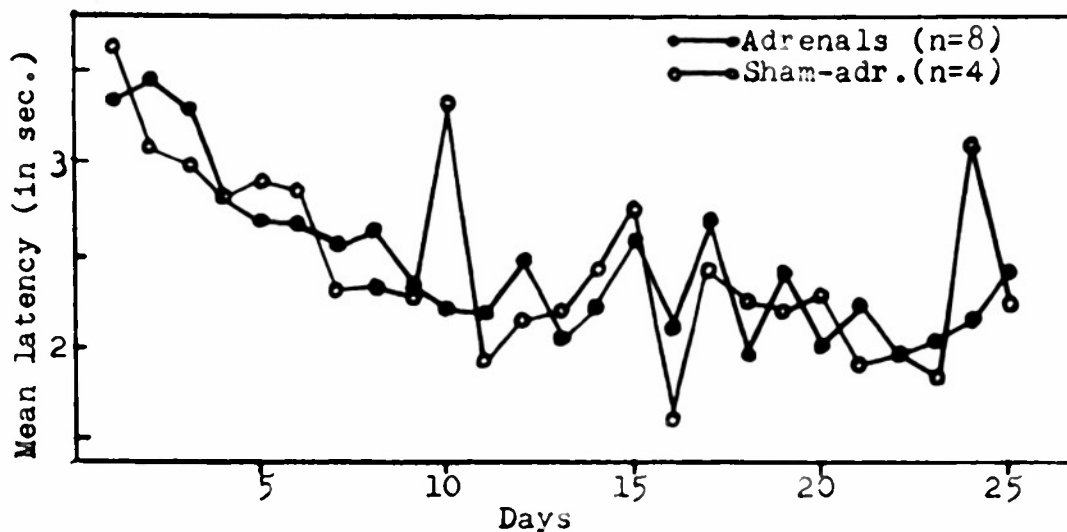


Figure 7. Mean latency of escape responses for daily blocks of ten conditioning trials. (Experiment 7).

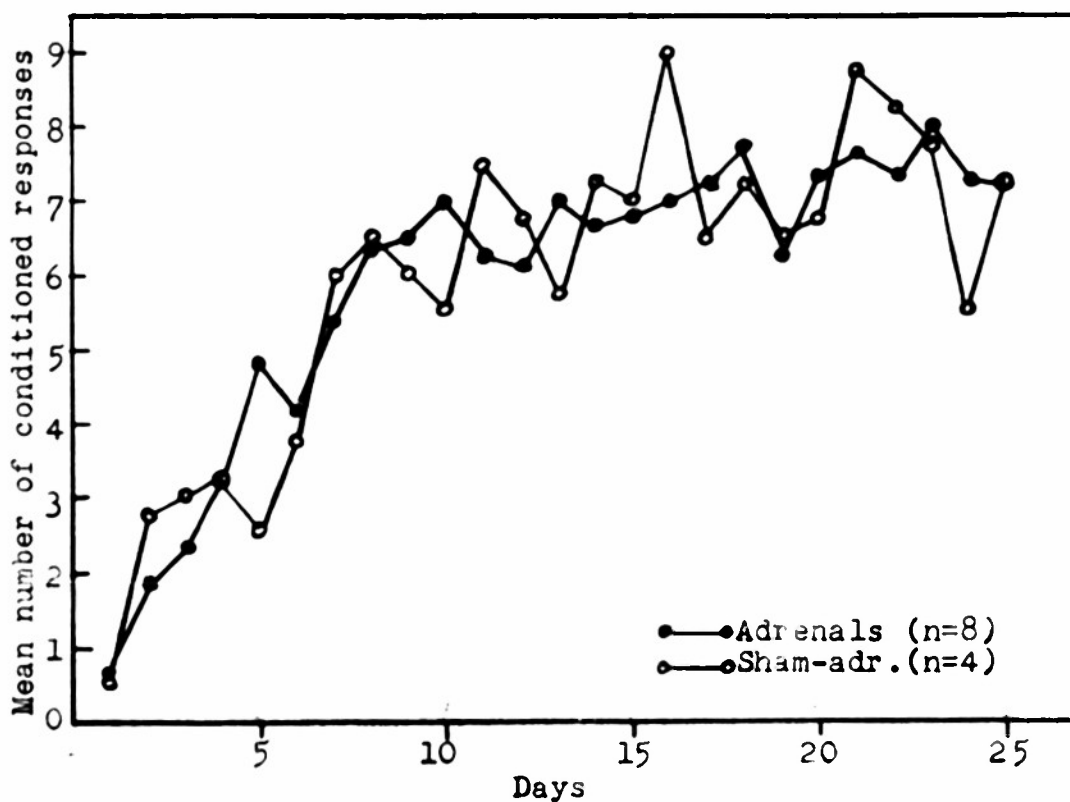


Figure 8. Mean number of conditioned responses for daily blocks of ten conditioning trials. (Experiment 7).

tion with noxious stimulation is used to account for the fact that animals receiving a series of such pairings (of light and shock) show an increase in the size of the startle response to a loud sound paired with the neutral stimulus but never paired with shock. A simplified version of the Brown-Kalish-Farber apparatus was used in these experiments to study the changes in amplitude of the startle response as a function of fear conditioning.

Test series were interpolated periodically during daily runs of shock-stimulated fear conditioning trials. Each test trial consisted in the substitution of a startle-provoking stimulus (loud sound produced by a Paper-Popper Pistol) paired with the same light-stimulus regularly preceding shock in place of the unconditioned stimulus. Response amplitude was measured in units of vertical displacement of a small platform upon which the animal is confined. A lever attachment to the platform records both shock and startle displacements on a kymographic record which are then read with a 1/50th-inch rule.

The experimental procedure consisted of an habituation day in which the startle stimulus (sound) was presented four times and a 2-second shock stimulus twice, in the order: sound, sound, shock, sound, shock, sound. The CS (light) was not used on this day. This was followed by four training days

on each of which ten trials were presented, seven of these being paired CS (light) - UCS (shock) trials and three CS (light) - startle stimulus (sound) test trials, the latter presented as the fourth, seventh and tenth trials for all series.

The first of these experiments compared hypophysectomized rats and sham-operated controls. The control group showed a significantly greater gain in amplitude of response over trials than did the hypophysectomized animals ( $P = < .06$ , both tails).  $t$ -tests for gains within each group showed a significant increase in amplitude of startle response for the control group ( $P = < .01$ ), whereas the change in amplitude of this response for the hypophysectomized group failed to approach significance (see Figure 9). No differences were found between the groups (see Figure 10), with respect to the amplitude of the response to the unconditioned stimulus (shock). This finding would seem to indicate that the inferiority of the hypophysectomized group can not be attributed to debility in these animals.

The second experiment of the three compared hypophysectomized-adrenalectomized rats and double-sham-operated controls. Evaluation of gains over trials between the groups reveals a significantly greater gain in amplitude of startle for the control group over the double-operated animals ( $P = < .04$ ).  $t$ -tests for gains within each group showed a significant

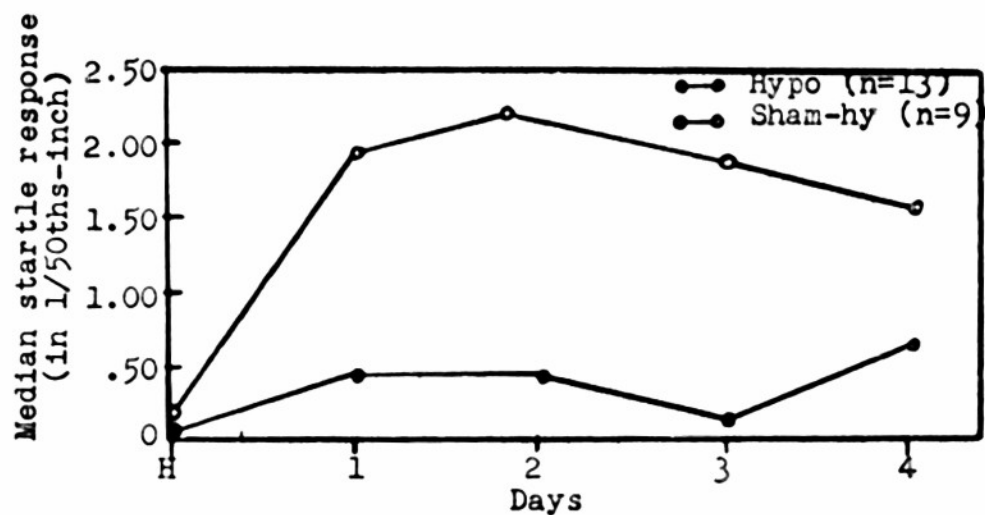


Figure 9. Medians of mean magnitudes of startle response (1/50th inch) for blocks of three test trials during habituation and conditioning in Experiment 8.

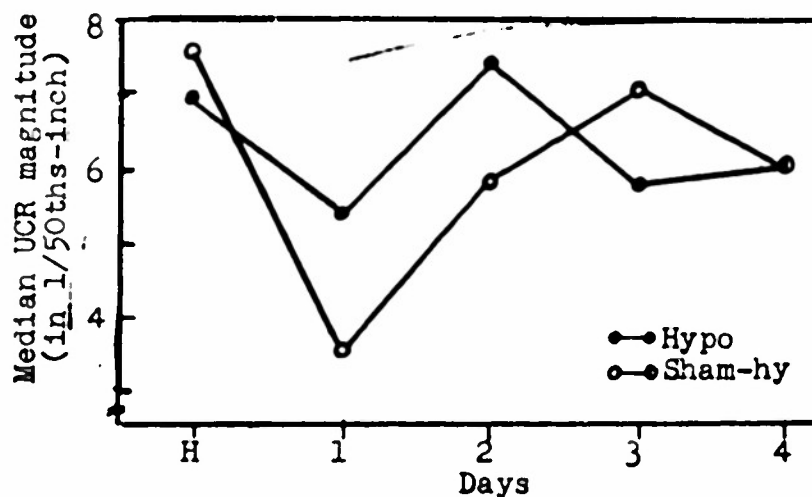


Figure 10. Medians of mean UCR (shock) magnitudes for blocks of two trials on Habituation Day and seven trials on Conditioning Days in Experiment 8.

increase in amplitude of startle response for the control group ( $P = < .01$ ), whereas the change in amplitude of this response for the hypophysectomized-adrenalectomized group failed to approach a minimal criterion of significance (see Figure 11). The two groups did not differ with respect to mean amplitude of response to the shock stimulation (see Figure 12), again indicating that the capacity of operated animals to perform the requisite responses is not seriously impaired by the removal of the two glands.

The third of these experiments compared bi-laterally adrenalectomized rats with sham-adrenalectomized controls. The two groups were treated identically except for the substitution of a physiological saline solution for water in the home cages of the adrenalectomized animals. Both groups were run for a total of six days as compared with four in the previous two studies. No significant difference was found in a comparison of gains over trials between the groups (see Figure 13). The gain in amplitude of startle over days for the control group was not significant ( $P = .20-.10$ , both tails), primarily because of the large individual differences within the group (e.g., one animal started with a very large startle on the first test trial, dropped on the second day and then gained steadily, but failed to show a first day - last day gain). The change in amplitude of this



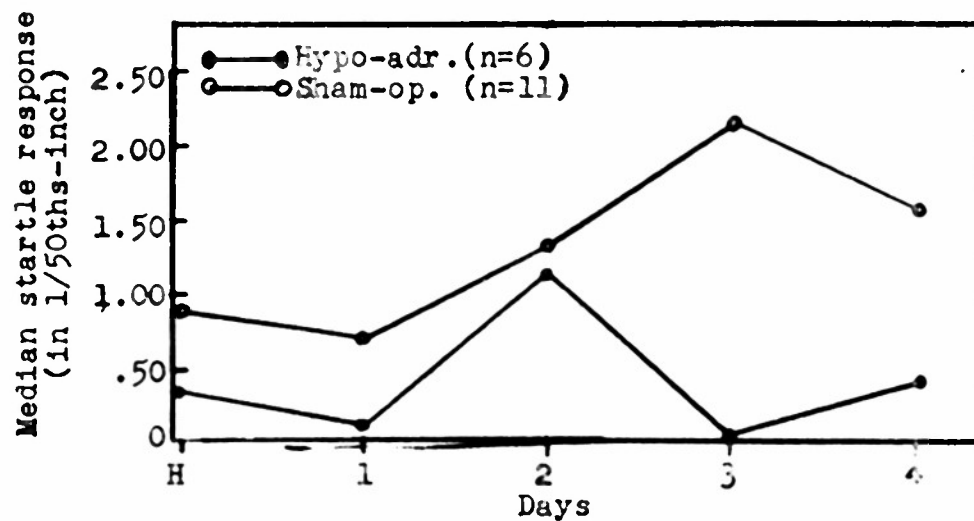


Figure 11. Medians of mean magnitudes of startle response (1/50th inch) for blocks of three test trials during habituation and conditioning in Experiment 9.

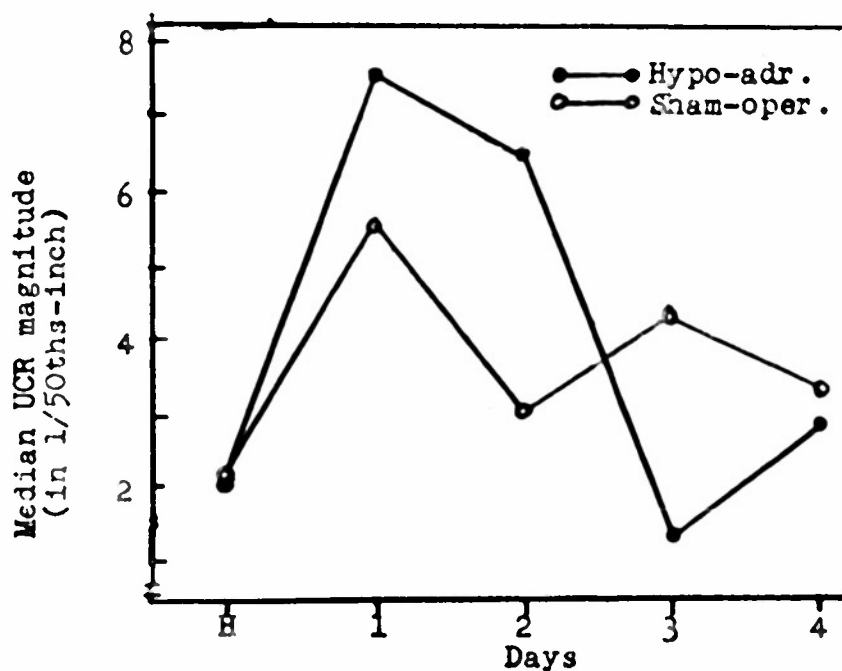


Figure 12. Medians of mean UCR (shock) magnitudes for blocks of two trials on habituation day and seven trials on conditioning days in Experiment 9.

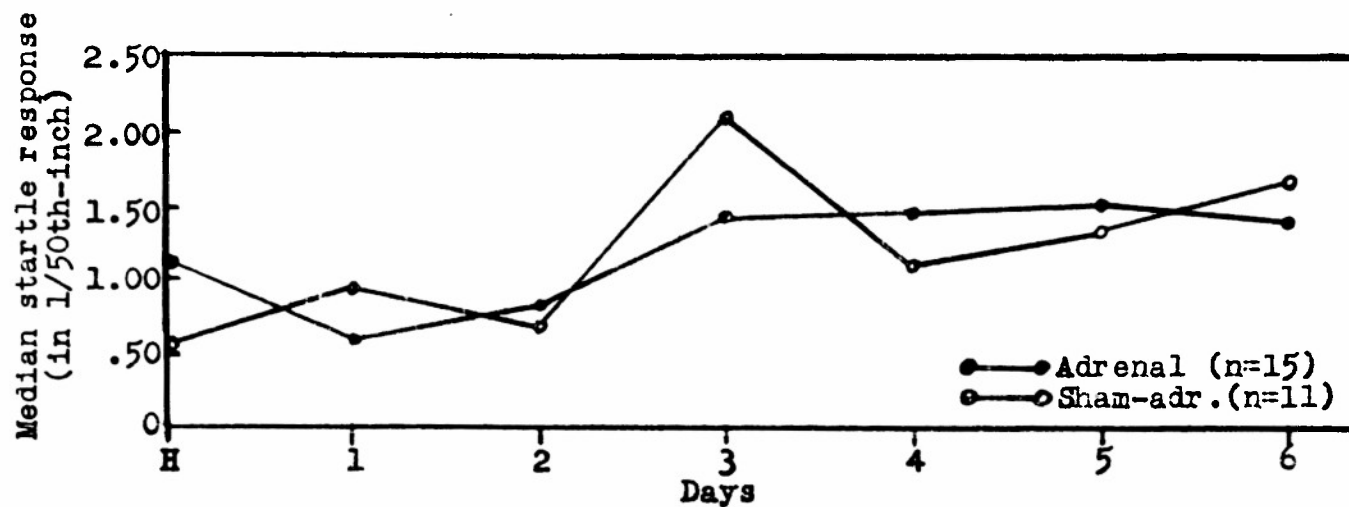


Figure 13. Medians of mean magnitudes of startle response (in 1/50th-inch units) for blocks of three test trials during habituation and conditioning in Experiment 10.

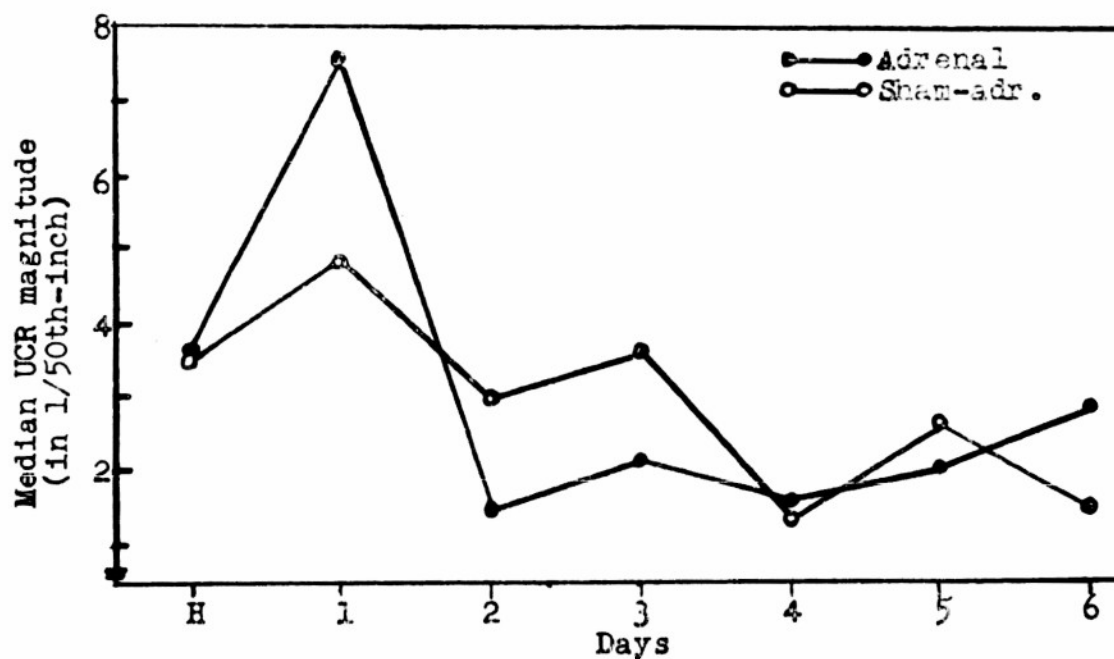


Figure 14. Medians of mean UCR (shock) magnitudes for blocks of two trials on habituation day and seven trials on conditioning days in Experiment 10.

response over days for the adrenalectomized group was significant, however, ( $P = < .05$ ). The two groups did not differ with respect to mean amplitude of response to the unconditioned stimulus (see Figure 14).

Examining the three studies together, it appears that all groups are capable of responses of the same order of magnitude (as seen in the comparisons of displacement responses to shock stimulation), but only in the adrenalectomized rats, of the three insulted groups, is there evidence of the acquisition of a conditioned fear response. Hypophysectomized animals, with or without their adrenal glands, fail to acquire such a response.

#### Experiment 11.

An attempt was made to study avoidance learning in the Miller-Mowrer shuttle box without forced dislodging of animals attempting abortive<sup>7</sup> responses. Meaningful data could not be obtained because a large number of animals in both hypophysectomized and sham-operated groups did develop such non-running escape responses to shock. One observation of interest in this study was the apparently greater

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<sup>7</sup> Abortive responses here mean any position(s) taken by the animal which led to successful escape from shock without moving across the box. Despite care in construction (which eliminated grid-shorting by the animal) rats did manage to straddle bars and cling to wall surfaces in such a way as to avoid shock.

development of "emotional" responses (squealing, gasping, heavy breathing) in the hypophysectomized animals as compared with sham-operated controls. "Emotional" responses did not appear to be related to the frequency of successful escape or avoidance responses to shock (either running or abortive), however.

#### Experiment 12.

The next study utilized the apparatus described by Miller (9) to measure acquired fear. This apparatus is of approximately the same size and shape as the Miller-Mowrer shuttle box, but is separated more distinctly into two compartments, only one of which (the left) has a grid floor. The two compartments are divided by a wall which extends two-thirds of the way to the floor, leaving a doorway the width of the compartment and approximately three inches high for the animal to move through. The walls of the grid-floor compartment were painted white while the "safe" chamber walls were painted black, adding to the differentiability of the two sections. Entry into the shock chamber was through the hinged roof. The grid floor was so arranged that the weight of the animal being dropped upon it released a microswitch which charged the grid and started a timer. A floor-switch in the doorway of the "safe" chamber automatically stopped the timer, thus recording latency for each

response.

Two groups of rats, one hypophysectomized and the other sham-operated, were given 100 trials over five days in which they were dropped onto the charged grid and permitted to escape through the door into the safe compartment. Two "test" trials (in which the procedure was the same but the shock was omitted) were given randomly during the second and fourth quarters of each daily run of twenty trials. Starting with the sixth trial on the sixth day, shock was turned off and remained off for the duration of the experiment. A maximum of sixty seconds was permitted for any one non-shock trial, the animal being removed from the grid chamber at the end of this period if no crossing response occurred.

A comparison of shock-escape latencies (see Figure 15) showed no significant differences between the groups, although the operated rats were somewhat slower on the first day of shock-escape training. Latencies on non-shock test trials were not significantly different during training, although pre-training latency measures and those for the first test trial on the first training day showed a longer mean latency for the hypophysectomized group than for the controls. A difference in this same direction appeared during the last three test trials of the training period, although, again, the differences were not statistically signi-

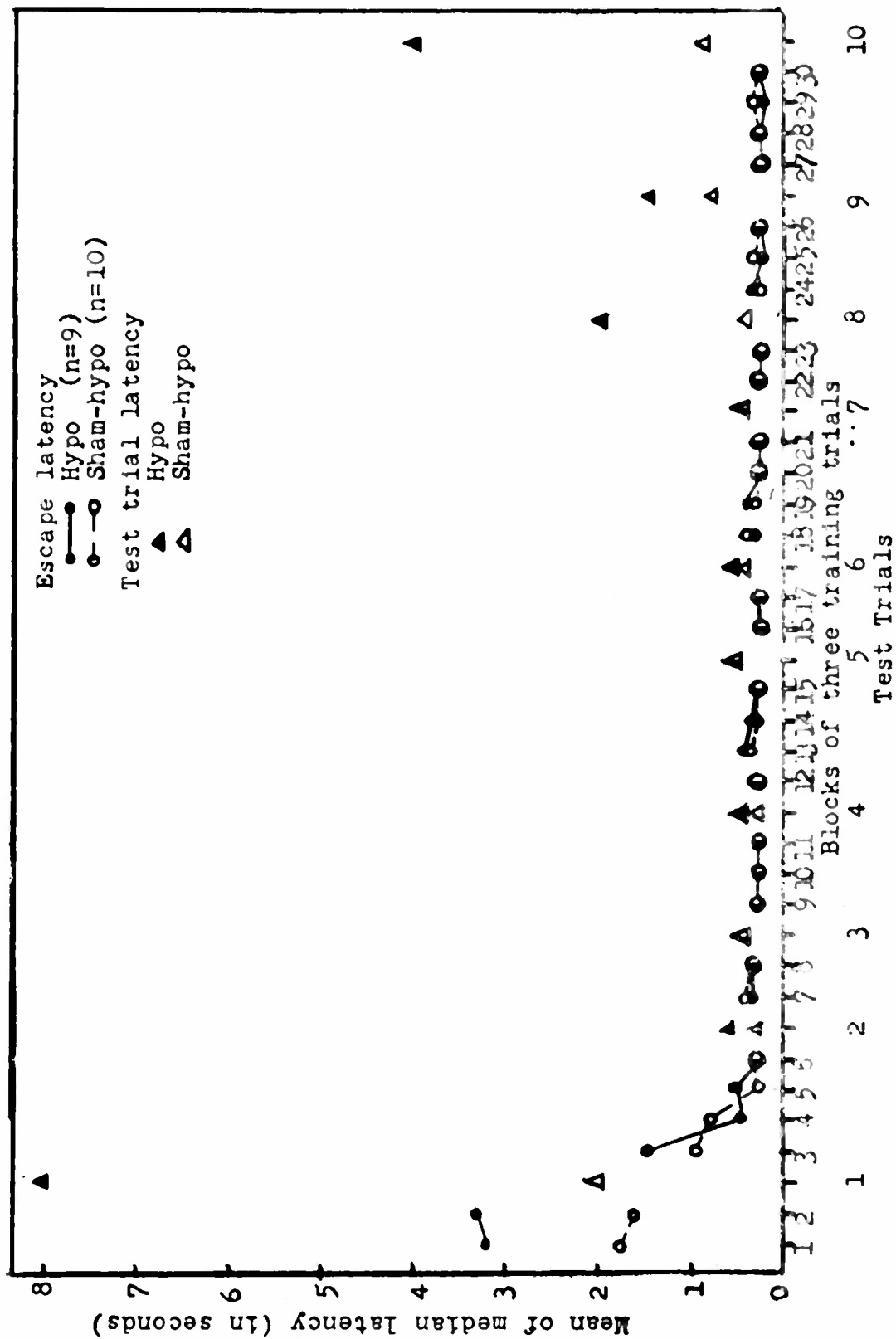


Figure 15. Means of median latencies of escape response in blocks of three training (90 trials in all) and median latencies for each of ten test trials (Experiment 12).

ficant.

If no fear response to the grid chamber is acquired during the shock-escape training, it would be expected that crossing responses would not occur once the shock had been removed from the situation. The latency differences in the test trials on the first and last training days were in a direction suggesting that this might be the case for the hypophysectomized animals, but the similarity of the short-latency test trial responses during the middle three days of training for the both groups seems to indicate that both groups have acquired a conditioned fear response of the same order of magnitude.

Examining the performance of the two groups on the "extinction" series (Figure 16), it is found that they do not differ significantly on the number of non-shock trials required to reach a criterion of five successive no-response trials, nor were they significantly different in a day-to-day comparison of mean per cent of non-response trials during the five day extinction period.

As the latency of the first crossings without shock on two successive pre-training days showed the hypophysectomized group to be approximately ten times as slow as the control animals in this study, the failure of the operated group to extinguish more rapidly once shock was removed would seem to suggest the acquisition of the fear response

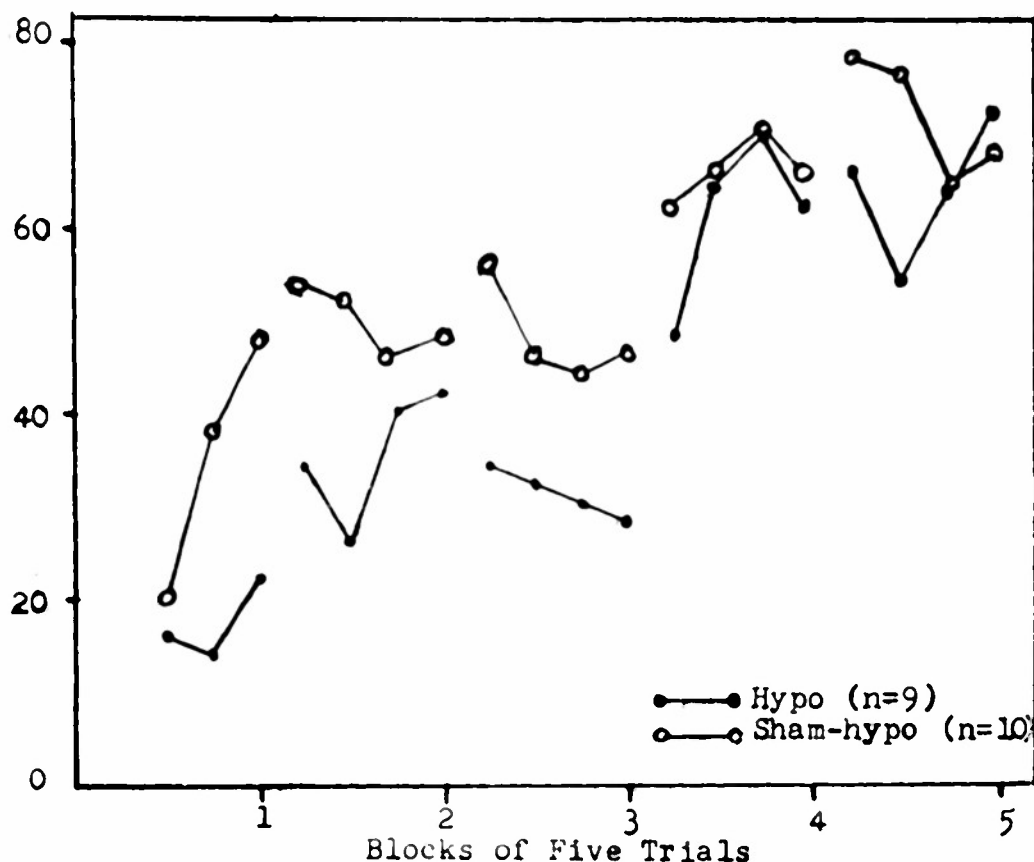


Figure 16. Mean per centage of failures to respond for blocks of 5 extinction trials. (Experiment 12)

in this group, as well as in the controls, during the shock-escape training. This evidence clearly contradicts the major hypothesis and is in contrast to the findings of the other studies in this series.

#### Experiment 13.

To vary some of the conditions of avoidance learning, a preliminary study was conducted, using normal animals, to determine the feasibility of training an avoidance response to light as an unconditioned stimulus in an arrangement similar



to the shock-avoidance situation of the Miller-Mowrer shuttle box. A buzzer stimulus was used as the CS and a 31.167 millilampert light<sup>8</sup> was substituted for the shock UCS. No avoidance conditioning was obtained in 110 trials, nor, for that matter, was there any sizeable decrease in latency during the training procedure. This method was then abandoned in favor of the one to be described in Experiment 14 below.

#### Experiment 14.

Light-escape and light-avoidance were tested in another experiment with hypophysectomized and sham-hypophysectomized rats by measuring the latencies of crossings from light to dark and from dark to light in an oblong chamber comparable in size with the Miller-Mowrer box. No buzzer was used in this experiment. The oblong box is pivoted at the center so that the weight of the animal moving from one end of the box to the other would tilt the box sufficiently in the direction of the movement to open or close a microswitch placed under one end of the box, thus permitting the recording of latency and number of crossings automatically.<sup>9</sup> Movement

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<sup>8</sup> A value found by Hanson (5) to produce a stable light-aversive response.

<sup>9</sup> The apparatus used in this study was the one described by Zeaman and Radner (19). In a later study this apparatus was modified to permit the running of eight animals simultaneously and the recording of direction and latency of movement kymographically.

across the box in one direction caused the light to be turned on in the box, whereas crossings in the other direction automatically turned the light off. Each animal was given a 15-minute period in the box on each of eight successive days. A 5-minute period in the light and three 15-minute periods in the dark were given on the four successive days preceding the eight test days. The three days of pre-test exposure to the box in the dark were used as a basal activity measure for later equation of latencies. Each animal was trained against initial position preference, the side preferred during pre-training being made the light-on side for that animal during training.

Results of this experiment were as hypothesized. Sham-operated controls showed a mean dark-to-light latency increase over the eight day training period, whereas the hypophysectomized animals actually showed a mean loss during this period (see Figure 17). The gain within the sham-operated group was significant at just short of the .05 level (both tails), whereas the change within the hypophysectomized group did not approach statistical significance ( $t = < 1$ ). The difference in gains between the groups is highly significant ( $P = < .01$ ). The normal animals thus showed evidence of learning to anticipate the coming on of light stimulation as a result of crossing in the dark, the operated animals giving no evidence of having acquired this

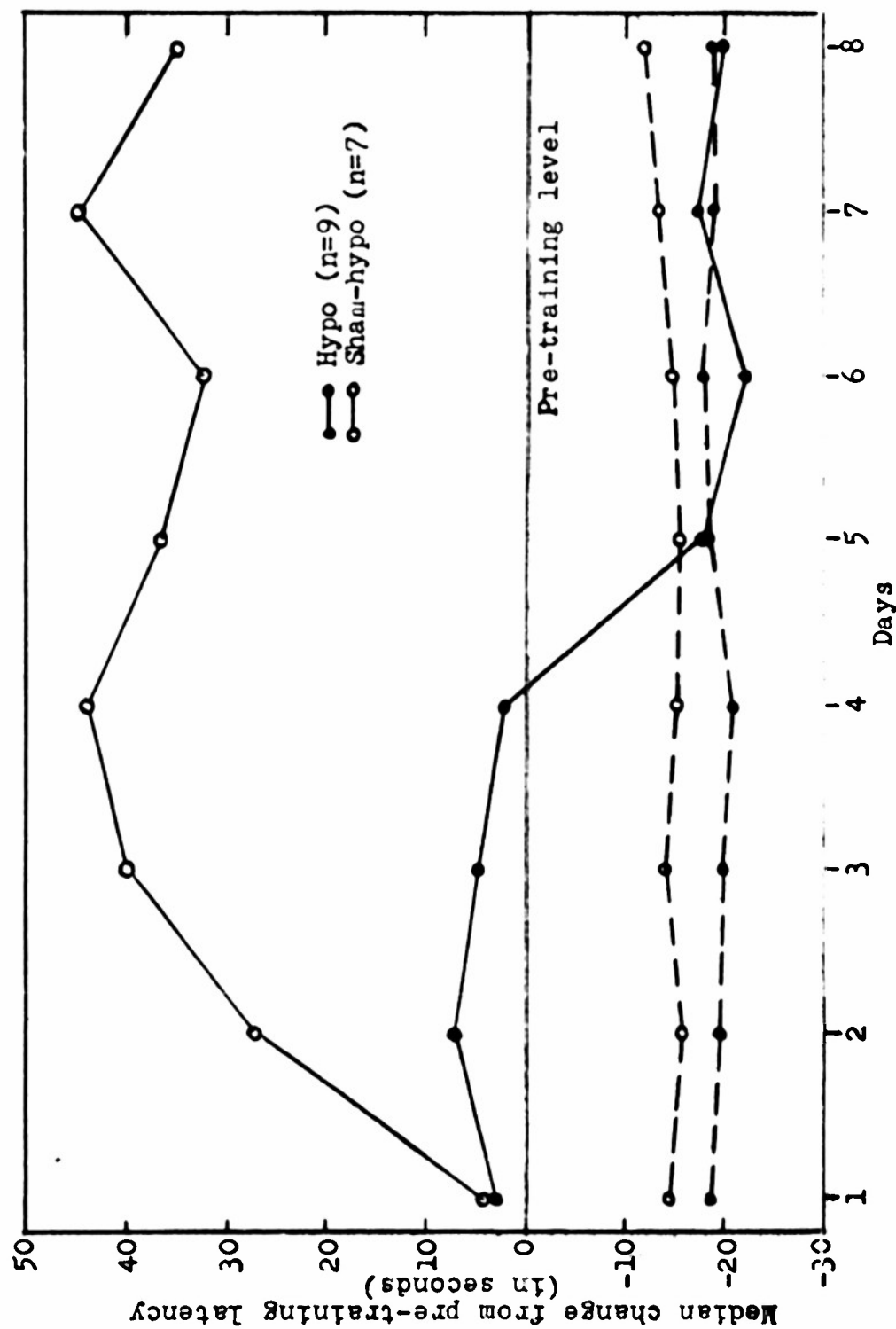


Figure 17. Medians of mean changes from pre-training level in latency per crossing from dark to light (solid line) and from light to dark (broken line). Experiment 14.

anticipation. The groups did not differ in their light-to-dark latencies, suggesting that light was an aversive stimulus of approximately the same moment for both groups and indicating again that failure to learn an avoidance response is not attributable to general debility in the operated animals.

#### Experiments 15 and 16.

Several additional exploratory procedural studies were undertaken with normal animals in the Miller-Mowrer shock-avoidance apparatus. One study compared two levels of shock ( $.8 \pm .1$  ma vs.  $.2 \pm .05$  ma) in an attempt to determine if the amount of shock heretofore employed could be reduced. The findings suggested that less efficient and more erratic conditioning resulted with smaller shock.

A study of two distributions of practice (10 vs. 20 trials per day) showed the latter to produce a somewhat more rapid decrease in latency and a somewhat more rapid rate of acquisition of conditioning with no apparent decrement due to the greater massing of trials.

#### Experiment 17.

One more attempt was made to replicate the findings of Applezweig and Baudry (1). Hypophysectomized and sham-operated rats, half of which were given daily injections of .5 mg ACTH; were given 20-trial runs on alternate days

for a total of 200 trials. Animals not receiving ACTH were given equivalent injections of .09% physiological saline solution. Although preliminary study seemed to indicate that conditioning proceeded relatively smoothly in this apparatus, an examination of the data of this experiment revealed that only 40 per cent (2 of 5) of the sham-operated, saline-injected control group met the criterion of 50 per cent response in any one day. It is possible that the alternate day running schedule was responsible for this failure to condition more adequately. Of the sham-operated animals receiving ACTH,<sup>10</sup> 67 per cent (4 of 6) reached this criterion. The two hypophysectomized groups produced 33 per cent (3 of 9) and 20 per cent (2 of 10) learning in the ACTH and saline groups, respectively.

The bimodal distributions within the groups precludes statistical analysis or any definitive interpretation. However, the comparative data of the four groups (see Table 2) suggests the slight superiority of the two ACTH-treated groups over their saline-injected controls. The data are also in agreement with the consistent previous findings of the inferiority of hypophysectomized rats to sham-operated normals in this avoidance situation.

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<sup>10</sup>ACTH dosage used in Experiments 17 and 18 based on recommended animal dosage for Armour LALA standard ACTH (Armour Laboratories).

Table 2. Median number of conditioned responses  
for blocks of twenty test trials

Subj.	Sham-op. saline	Sham-op. ACTH	Hypophy. saline	Hypophy. ACTH
1	13	18	9	9
2	10	13	6	8
3	0	8	4	8
4	0	3	2	4
5	0	2	1	1
6		0	1	0
7			1	0
8			0	0
9			0	0
10			0	

#### Experiment 18.

An attempt to repeat the light avoidance study (experiment 14) and extend it to include ACTH-treated animals was frustrated by the performance of the sham-operated, saline-injected control group which spent over 90 per cent of its time in the dark during the first test day. There was thus little possibility of improvement during the eight day experimental period. The sham-operated, ACTH-injected animals and the hypophysectomized rats receiving daily ACTH injections both showed some gain over days, whereas the saline-

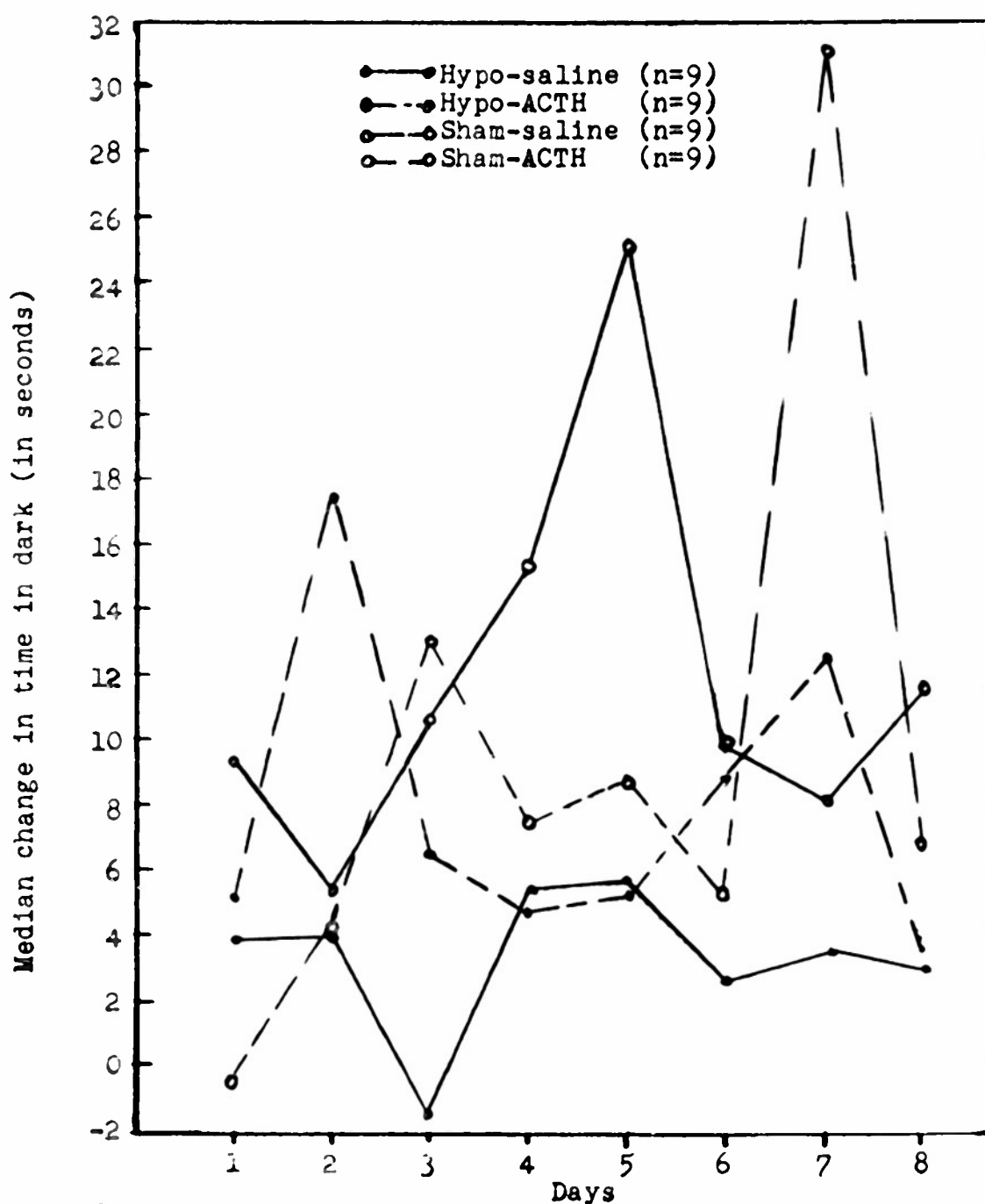


Figure 18. Median change from pre-training level in mean time per crossing from dark to light. (Experiment 18)

injected hypophysectomized group showed no improvement during the same period, in this respect showing similarity to the performance of the like-operated group in Experiment 14.

## Discussion of Results

### A. Food and Water Consumption.

It is clear from an evaluation of the data on food and water intake of normal, hypophysectomized and adrenalectomized<sup>11</sup> animals that these groups are not directly comparable with respect to either food or water consumption levels under routine laboratory household conditions. It would therefore not seem reasonable to use either hunger or thirst as motivating or reward conditions in studies comparing the learning of these groups.

### B. Activity.

Four independent measures of activity level were used during these studies. Behavior samples were taken in a rotating activity wheel, measurements were made of spontaneous (between trial) crossings in shock-avoidance training, and records were kept on latency of escape responses and of startle responses to shock, and of pre-training crossings under both all-light and all-dark conditions in light avoidance experiments.

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<sup>11</sup> Adrenalectomized rats, of course, required a physiological saline supplement to maintain life in the absence of the adrenal glands and were thus not directly comparable to the other groups with respect to water intake. Their liquid intake was considerably greater than that of any other group, as might have been expected.



The findings with respect to activity differences are not consistent over all situations studied. Within-group variations for all groups are extremely large under all conditions. Hypophysectomized animals appeared to be less active than normals under some circumstances (pre-training in the Miller box, pre-training in light and in dark in light avoidance situations) and about as active as control animals under other conditions (activity wheel, shock escape, spontaneous crossings during avoidance training, startle reaction to shock). The activity data are consistent for situations in which shock was employed, where no significant differences between groups were found, whereas non-shock situations produced differences favoring normal animals at some times and produced no differences at others. Under no conditions were the hypophysectomized animals found to be consistently more active than normals.

Adrenalectomized rats showed no clear differences, when compared with normals, with respect to activity as variously measured.

### C. Animal Weight.

Hypophysectomized animals, whether maintained with ACTH or not, consistently failed to show weight gains throughout the various study periods. Control animals and adrenalectomized rats (the latter maintained on .09% saline solution) showed normal growth curves.

#### D. Emotionality.

During the course of all of the studies in this series, the experimenters reported the presence of commonly accepted manifest signs of "emotionality" in operated as well as in intact animals. Reference is here made to such indices as gasping, squealing, freezing, heavy breathing, urination, defecation, washing behavior, and attempts to escape from the apparatus. These responses occurred not only in the presence of shock but during the presentation of the conditioned stimulus and in exploratory and box habituation periods as well. There was clearly no marked deficit in this type of behavior observed in either hypophysectomized or adrenalectomized animals.

#### E. Escape Learning.

For both shock and light escape situations, improvement in efficiency of the escape response (in the presence of noxious stimulation) of hypophysectomized and adrenalectomized groups was comparable to that of intact animals.

#### F. Fear Conditioning.

1. Adrenalectomized animals. The capacity to acquire a conditioned fear response, as manifested by increased sensitivity to startle stimulation, is not impaired by complete bi-lateral adrenalectomy in rats, nor is there any deficiency in the development of an instrumental avoidance response to

shock in these animals, as compared with sham-operated normals.

2. Hypophysectomized animals. The capacity to acquire a conditioned fear response, as measured by increased sensitivity to startle stimulation, is impaired by removal of the hypophysis in the rat, and is likewise deficient in hypophysectomized rats whose adrenals have been removed in addition.

The rate of acquisition of conditioned instrumental avoidance responses, for all studies in which rate was measured during acquisition, is slower for hypophysectomized animals when compared with sham-operated controls. In addition, a smaller proportion of the hypophysectomized animals studied attained the learning criterion as compared with the proportion of control rats reaching this criterion within the fixed periods of study (usually 200 trials). In the one study employing an extinction measure of learning, no significant differences were found between hypophysectomized and control groups. Since this particular study used only two groups, both given an equal number of training trials, no definitive conclusion can be drawn with respect to the rate of acquisition in this one experiment.

G. Effects of exogenous ACTH administration.

1. Hypophysectomized animals. In four studies of

avoidance learning, exogenous administration of ACTH, in the amounts used, either slightly facilitated learning or produced no effect in hypophysectomized rats. In no case was the performance of an hormone-treated group inferior to that of saline-injected hypophysectomized controls.

Exogenous administration of ACTH, in the amounts used, had no apparent effect upon activity level of hypophysectomized rats, under either of the two conditions in which it was measured for these animals.

2. Normal animals. Equivocal findings must be reported regarding the influence of exogenously-administered ACTH on normal rats. Applezweig and Baudry (1) have reported a slight, though not statistically significant, depressing effect upon the avoidance learning curve with daily administration of .5 mg ACTH to normal animals. In the four studies in this series in which ACTH was administered to normal animals learning an avoidance response, it was found that a daily amount of approximately 16 micrograms had no apparent effect upon learning, whereas daily injections of 1.5 to 2.0 mg appeared to depress the learning curve for normal rats slightly below that for saline-injected normal controls. In the two later studies of ACTH effect upon avoidance learning in normal rats, daily injections of .5 mg of the hormone seemed to produce a slightly accelerating effect.

A more systematic study of ACTH dosage in these situations for both normal and hypophysectomized animals is clearly indicated.

### General Discussion

It may be recalled that these studies were undertaken to investigate the hypothesis that an intact pituitary-adrenal system is necessary for the acquisition of avoidance responses mediated by fear. If this hypothesis is correct, interruption of this system by removal of either the pituitary or the adrenals should interfere with this type of learning. It is clear from our findings that this is not the case. Interference, but not complete disruption, is produced by hypophysectomy, while adrenalectomy does not affect the acquisition of fear-motivated responses. Attempted replacement of the pituitary function in this system by exogenous administration of the adrenal cortical trophic hormone of the pituitary was only partly successful in reducing this interference.

It thus appears that the pituitary may play a role in this type of learning, whereas the adrenals do not.

The case for anxiety, or fear, as a mediating condition in the development of avoidance learning was most clearly presented by Mowrer (10) and has been further elaborated by many others (e.g., Mowrer and Lamoreaux (11), Miller and Dollard (8), Miller (9)). Mowrer speculated that the mechanism underlying anxiety would be found outside the central nervous system. Solomon and Wynne (17) attempted to test this peripheral theory of anxiety by training dogs in an avoidance situation after blocking the splanchnic nerve. They were

able to demonstrate a reduction in the rate of acquisition of avoidance responses, and concluded that although the autonomic nervous system plays a part in avoidance conditioning it is not absolutely necessary for it. The findings of the present investigations would rule out another possible "basic" mechanism outside of the central nervous system, namely, the adrenal glands. The results of the experiments reported here do, however, offer support for the belief that extra-central nervous system components may play a role in avoidance learning.

The present research concerned itself with the role of the pituitary-adrenocortical system in this type of learning because of the extensive work of Selye and others on bodily reactions to stress (16) and the belief that the anxiety, or fear, response developed in the avoidance learning situation might well be related to the general alarm syndrome. That the picture is a highly complex one is evident from examination of the literature on stress (cf. Selye, 16).

The precise controlling mechanism for the pituitary itself is not yet understood (6), although it is now apparent that the role of the adrenal medulla may not be critical for this particular stress response system (4,7).

The findings of the present studies with respect to adrenalectomized animals would, in a similar manner, as has been indicated above, tend to de-emphasize the role of the adrenal medulla in fear reactions. This would seem to be true

for the adrenal cortex, too. The pituitary itself, however, appears to be involved in the anxiety mechanism, insofar as the course of avoidance learning is altered by hypophysectomy.

### Some Alternative Hypotheses

1. The pituitary-adrenocortical system is necessary for avoidance learning. The major hypothesis which these studies set out to test was that interference with the pituitary-adrenocortical system would interrupt pain-expectancy, or anxiety, and thus interfere with avoidance learning. It is now clear that the hypothesis, in its original form, must be abandoned. If the intactness of the entire system were important, the removal of the adrenal glands, as well as of the hypophysis, should have served to disrupt this training. We have found that the removal of the pituitary does appear to decrease the efficiency of this learning, but the removal of the adrenals has no influence on its course. Clearly, the "anxiety" involved here cannot be traced to any function of the adrenals, and, by implication, is not a function of the action of ACTH upon the adrenals. The continued presence of many of the usual manifest signs of "anxiety" in both hypophysectomized and adrenalectomized animals would also suggest that an all-inclusive anxiety mechanism has not been isolated. At least two alternative possibilities seem to exist here. First, that the release of ACTH into the blood produces a change or changes somewhere else in addition to its trophic

influence upon the adrenal cortex, or, secondly, that it is not the pituitary-adrenal system but some other pituitary system which is involved. Unfortunately, the ACTH studies here reported are not sufficiently clear to permit determination of this issue. The fact that some improvement was noted in the avoidance learning of hypophysectomized rats receiving ACTH (compared with those not receiving this hormone) would suggest that the first of these alternatives may well be worth further exploration before moving on to the second.

2. Hypophysectomy produces general debility. There is little question that hypophysectomy interrupts a number of vital life processes in addition to the pituitary release of the adrenal cortical trophic hormone. The findings of inferior avoidance learning in hypophysectomized rats might then be explained in terms of an hypothesis of general debility in these animals. However, measurements of activity in non-avoidance situations and of escape from present noxious stimulation reveal that these animals are as able as normals to make the motor responses required of them. As it is only in situations calling for the anticipation of noxious stimulation that they are deficient, a general debility hypothesis would appear to be inadequate to a differential prediction of response in non-avoidance and avoidance situations.



3. Alternate pathways. That a particular function may have primary dependence on a given pathway does not rule out the possibility that interruption of this pathway will lead to the development or refinement of an alternate medium of action. The capacity of an insulted organism to compensate for sometimes quite severe disturbances to its homeostatic mechanisms is well known at all levels of biological science, and needs no elaboration here. That some organisms survive stresses which overwhelm others may be a function of the ease with which auxiliary mechanisms can be brought into action to substitute for those destroyed or disturbed. The survival of some bilaterally-adrenalectomized rats and not of others is an example in point.

The findings that autonomic blocking (17) and hypophysectomy (1, and present studies) both interfere with avoidance capacity raise the possibility that here, as in other areas, more than one peripheral system may be, or may become, the pathway for a given operation.

An "alternate pathways" hypothesis would perhaps suggest that the search for a basic peripheral mechanism is not as likely to produce definitive findings as might the exploration of higher order (hypothalamic, thalamic or cerebral) mechanisms.

### Summary and Conclusions

Eighteen studies undertaken to evaluate the role of the pituitary-adrenocortical system in avoidance learning are reported here. Studies of food and water intake, activity, shock- and light-escape and avoidance learning, and emotional conditioning were conducted with various combinations of hypophysectomized, adrenalectomized, sham-operated and normal albino rats. In four of these studies ACTH was administered in different amount to both hypophysectomized and sham-operated animals in an attempt to isolate the component of the pituitary response involved in avoidance learning.

It was found that the course of avoidance learning is interfered with by hypophysectomy, and that this interference could probably not be attributed to the general debility of the hypophysectomized organism. On the other hand, complete bilateral adrenalectomy has no apparent effect upon the rate or level of acquisition of avoidance learning. The two findings, taken together, lead to the rejection of the hypothesis that an intact pituitary-adrenal system is necessary for avoidance learning, and raise the possibility that pituitary action elsewhere may be an important factor in this type of learning.

ACTH-injections appeared to partially restore the learning capacity of hypophysectomized rats, although definitive conclusions must await studies of the effects of vary-

ing dosages of ACTH upon avoidance learning. If the accelerating effects of exogenous ACTH-treatment of hypophysectomized rats is confirmed, it would raise the additional possibility that ACTH may play a role in avoidance learning in some manner other than its trophic influence upon the adrenal cortex.

Some alternative hypotheses are discussed.

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